Kaolin Treatment of Sera for Removal of Nonspecific Inhibitors to Asian Strains of Influenza Virus.* (25545)

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Kaolin is used for removal of nonspecific serum inhibitors to the arthropod-borne viruses(1). It has also been used to remove nonspecific inhibitors to Asian influenza viruses from human and fowl sera and there is some evidence that treatment of sera with kaolin permits more cases of Asian influenza to be detected by the hemagglutination-inhibition (HI) test than treatment with trypsin (2). Kaolin has advantages over other substances used routinely for removal of nonspecific serum inhibitors to the influenza viruses, *viz.* trypsin, periodate and receptor destroying enzyme (RDE), in that it is easily available and relatively cheap. Furthermore, it does not have to be prepared fresh before use and treatment of a serum with kaolin can be accomplished within an hour, treatment overnight being unnecessary. The present study was undertaken to investigate the value of kaolin in routine diagnostic work with the Asian influenza viruses and to compare kaolin treatment with other technics.

Materials and methods. The HI test was carried out in the manner described by Jensen (3) with one modification, phosphate buffered saline (pH 7.2) replacing normal saline as diluent. Complement-fixation (CF) test was done by the method of Casals(4). Antigens were infected allantoic fluids. Sera were treated with periodate in the usual manner, trypsin(3), RDE(5) and kaolin and acetone (1). Treatment with kaolin(1) consists essentially in the following: to 1 volume of serum are added 4 volumes of borate-saline buffer at pH 9.0 and 5 volumes of a 25% suspension of kaolin† in borate-saline buffer. The mixture is held at room temperature for 20 minutes, centrifuged and the supernatant used.

Results. Acute and convalescent sera from 9 cases of Asian influenza, diagnosed by virus isolation during an epidemic in Trinidad in 1957, were treated by 3 different methods and tested by HI with the Asian strain A/Asian/Japan/305/57. Results are shown in Table I, which also gives the results of CF tests on the same sera. Kaolin treatment permitted 7 of the 9 proven cases of influenza to be diagnosed. Six cases each were diagnosed by the use of periodate and RDE.

In addition, sera from other cases diagnosed clinically as influenza but not confirmed by virus isolation were tested under the same conditions. Table II summarizes the results on all the sera tested, including those shown in Table I. When all the cases are considered, the results with kaolin compare favorably with the other methods.

The effectiveness of various technics in removing from normal fowl serum nonspecific inhibitors to 2 Asian influenza strains isolated in Trinidad is shown in Table III. Two other strains, B/GL/1739/54 and A/Denver/1/57, are included as controls. With normal fowl serum the best results were obtained with trypsin. Kaolin removed most of the inhibitor and gave better results than all of the other methods except trypsin.

Discussion. The methods used for removal of nonspecific inhibitors to influenza virus are not entirely satisfactory. In this laboratory trypsin treatment has not given reproducible results with the Asian strains. With other non-Asian strains treatment with trypsin has often been found to be disappointing, and there is no assurance that any of the treatments do not reduce specific antibody levels(6). Many of the Asian influenza isolates are very sensitive to nonspecific inhibitors in heated sera and treatment of sera with trypsin, periodate or *Vibrio cholerae* extracts

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† Obtained from Amend and Co., New York, N. Y. Specify acid-washed product.

TABLE I. Comparison of 3 Methods of Removing Nonspecific Inhibitors from Sera of Proven Cases of Asian Influenza.

Antigen	CF titers		HI titers					
			Sera treated with					
			Kaolin		Periodate		RDE*	
	Acute	Conv.	Acute	Conv.	Acute	Conv.	Acute	Conv.
A/Asian/Japan/305/57	8	128	10	80†	10	0	10	0
	0	64	0	20	10	20	0	40
	8	32	10	160	0	20	0	160
	0	8	10	40	0	20	0	10
	0	16	10	40	0	40	0	40
	0	32	0	20	0	20	0	20
	0	32	10	160	0	80	0	40
	8	128	20	40	0	40	0	20
	0	8	10	10	0	0	0	0

* Receptor destroying enzyme.

† Fourfold (= 2 tube) or higher rise in titer from acute to convalescent specimen accepted as evidence of infection.

TABLE II. Number of Cases of Influenza Diagnosed by the Hemagglutination-Inhibition Test When 3 Different Methods of Serum Treatment Were Employed.

Method of treatment	No. of cases tested	No. positive	% positive
Periodate	29	18	62
Kaolin	31	19	61
RDE	19	8	42

does not remove these troublesome inhibitors (7). As it is likely that the Asian strains will be prevalent for a number of years, alternative methods of removing inhibitors will be needed. These preliminary investigations suggest that kaolin may be useful in this respect. The possible use of kaolin for removal of nonspecific inhibitors to other myxoviruses should also be considered.

TABLE III. Treatment of Normal Fowl Serum by Various Methods for Removal of Nonspecific Inhibitors to Asian Strains of Influenza Virus Isolated in Trinidad.

Method of treatment of fowl serum	Virus strains			
	B/GL/ 1739/54	A/Denver /1/57	Trinidad 17359	Asian 17337
No treatment	0	0	40*	80
Inactivation at 56°C/30 min.	0	0	640+	640+
Kaolin	0	0	10	10
Kaolin + inactivation at 56°C /30 min.	0	0	20	20
Trypsin	0	0	0	0
Periodate	0	0	80	160
RDE	0	0	160	320
Acetone	0	0	160	80

* Reciprocal of maximum serum dilution which inhibited hemagglutination by 4 units of antigen.

During investigations on another outbreak of influenza in the summer of 1959 in Trinidad, the value of kaolin was again demonstrated. Six viruses were identified as Asian strains of influenza virus by the use of HI tests with kaolin treated fowl antisera. Periodate treatment failed to remove the inhibitor from the same antisera. Eleven cases diagnosed by the CF test and/or virus isolation were also diagnosed by the HI test using kaolin treated sera.

In view of the advantages of kaolin treatment over other technics further trials of this method are clearly indicated.

Summary. The use of kaolin for treatment of sera in diagnosis of Asian influenza cases by hemagglutination-inhibition test is reported. Comparison of kaolin treatment with other methods in common use is made. The advantages of kaolin treatment over other technics warrant further trial of the method.

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1. Clarke, D. H., Casals, J., *Am. J. Trop. Med. and Hyg.*, 1958, v7, 561.

2. Spence, L., Anderson, C. R., Downs, W. G., *Carib. Med. J.*, 1957, v19, 174.

3. Jensen, K. E., *Influenza,* in *Diagnostic Procedures for Virus and Rickettsial Diseases,* Am. Public Health Assn., N. Y. 1956.

4. Casals, J., *J. Immunol.*, 1947, v56, 337.
5. Zaghloul, I. S., Rizk, F., Kader, A., *Bull. WHO*, 1955, v13, 289.
6. World Health Org., *Tech. Rep. Ser.*, 1959, No. 170, 24.
7. Jensen, K. E., *J. Am. Med. Assn.*, 1957, v164, 2025.

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Effect of Ethionine on Amino Acid Composition of Rat Tumors.* (25546)

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We have shown that ethionine inhibited growth of 4 different tumors in rats but its effectiveness varied with strain and sex of host animals(4,12,14,18). Inhibition by ethionine of growth of tumors in mice has been reported from other laboratories(6,23, 26). Although many studies have been made (7,8,11,16,19,24,25,28-32), it is not clear whether ethionine inhibits incorporation of methionine *per se,* or of other amino acids, into proteins of the tumor or causes decreased growth of tumors by affecting adversely protein synthesis or other metabolic processes elsewhere in the body. It was reported(4,5, 12-15,18) that concentration of some "free" amino acids is decreased, and some increased, in liver of rats treated with ethionine. Our object was to determine whether composition of proteins, or concentration of "free" amino acids, in tumors of rats is affected by administration of ethionine.

Materials and methods. Young male and female rats (average weight 174 and 162 g, respectively) of Wistar strain, maintained on *ad lib.* diet of Purina Chow supplemented with weekly ration of fresh carrots, were divided into groups and treated as shown in Table I. Eighteen to 24 hours after last injection, the tumors were removed under ether anesthesia, freed as much as possible from connective tissue, rinsed with distilled water, blotted and weighed. The method of sample preparation was based on that of Schurr *et al.*(22). The viable tissue of each tumor was separated quickly from the necrotic portion, rinsed, and heated for 3 minutes in boiling water. The boiled viable tissues of each group were blotted free of water, separated from small amount of necrotic tissue remaining, cut into small pieces and pooled. The pooled samples were homogenized in Waring Blender and concentrated *in vacuo* to an appropriate volume depending on size of group sample. These suspensions were analyzed for dry weight, total nitrogen(9,27), total amino acids, and apparent free amino acids. Ash and mineral data obtained on these samples will be reported elsewhere. *Total Amino Acids*: Aliquots of tissue suspensions were refluxed in 8 N HCl for 24 hours and amino acids determined by microbiological assay methods previously described(2,3,14). *Apparent Free Amino Acids*: Aliquots of tissue suspensions were deproteinized with tungstic acid(22) and the amino acids determined by microbiological assay procedures described previously(2, 4,14).

Results. Data on number and sex of animals, tumors inoculated and material injected are given in Table I. Dosage of ethionine in females was reduced to ½ that used for males, to obtain a comparable degree of tumor inhibition in both sexes, since Dunn *et al.*(5,18) and Simpson *et al.*(25) reported that ethionine was more toxic, a more potent tumor-growth inhibitor, and affected liver composition to greater degree in female than in male rats.

Seven injections of ethionine inhibited growth of Sarcoma R-1 55% and 40%, and Walker carcinosarcoma 256 74% and 39% in males and females, (data omitted to conserve space). Degree of tumor inhibition ob-

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